Effects of Estrone on Incorporation of ^{14}C -Glutamic Acid in Rat Blastocyst during Delayed Implantation

Hirotsada TSUJII, Shichiro SUGAWARA and Saburo TAKEUCHI

Department of Animal Science, Faculty of Agriculture,
Tohoku University, Sendai-shi

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Abstract The effect of a single dose of estrone on the incorporation of ^{14}C -glutamic acid of delayed rat blastocysts was studied with liquid scintillation technics. Delayed implantation was induced by ovariectomy of the pregnant rats on day 3 of pregnancy (day 1=vaginal plug) and daily injections of 4 mg of progesterone. A single dose of 4 μg estrone was injected day 5 (102 hr) and day 8 (172 hr) of pregnancy, and the blastocysts were obtained from the animals killed at 6, 12, 24 and 30 hour after estrone administration. A single dose of 4 μg estrone had an effect on the incorporation of ^{14}C -glutamic acid during delayed implantation, and the incorporation increased rapidly until 24 to 30 hour after estrone treatment, but these incorporations were lower than the values observed in intact normal pregnancy. The addition of glucose or lactate as an energy source had an effect on the incorporation of ^{14}C -glutamic acid during delayed blastocyst.

We have previously studied on the protein synthesis in rat embryo during the cleavage stage¹⁻⁴). It has been confirmed in these investigations that the incorporation of ^{14}C -leucine and ^{14}C -glutamic acid into the protein fraction increased by addition of energy sources, such as glucose, lactate, pyruvate or succinate.

Protein synthesis in embryos have been demonstrated by the autoradiographical method in the mouse⁵⁻⁸) and rat^{9,10}) blastocysts during delayed implantation. In the present study, the effects of estrone on incorporation of ^{14}C -glutamic acid in the blastocyst during the delayed implantation was investigated by the liquid scintillation spectrometer. Glutamic acid was chosen, because the content of glutamic acid was highest in the free amino acids of uterine fluid in the pseudopregnancy and preimplantation stage¹¹).

Materials and Methods

Adult virgin female rats of the Wistar strain, weighing from 190 to 250 g were used.

The animals were divided into five groups, consisting of four or five sub-groups. Group 1 (control) consisted of five sub-groups of intact, pregnant animals. Animals from each sub-group were killed at various times after fertilization was same as Group II and III. Four o'clock of the morning following proestrus was considered to be the time of fertilization and zero hour of pregnancy. The treatment groups (Group II-V) consisted of pregnant rats in which artificial delayed implantation was induced by the method described by COCHRANE and MEYER¹²). The experimental design is shown in Fig. 1. The day of detection of spermatozoa in the vaginal smear was day 1 of pregnancy. The basic medium used in the experiment was Ca-free Krebs Ringer phosphate buffer at pH 7.4 (K.R.P.). The blastocysts were flushed from

Fig. 1. Experimental design for treated rats. Each day begins at midnight. Group II and IV received only progesterone treatment. Group III and V received progesterone plus estrone treatment.

the uterine horns. The blastocysts collected in a watchglass were examined under a microscope and the normal blastocysts were transferred into 0.1 ml of solution in a centrifuge tube. The number of blastocysts used for measurement was 25 per one sample. After preincubation at 37.5°C for 5 minutes, 0.1 ml K.R.P., glucose or lactate and 0.5 μ ci/ml universal labelled ^{14}C -L-Glutamic acid (spec. act. 0.11 mci/mm) were added at the same time. These samples were incubated for 0 and 1 hour. The final concentration of glucose was 10^{-2}M and sodium lactate was $5 \times 10^{-2}\text{M}$, which are generally used for mammalian eggs^{3,11,13}. After each incubation time, the reaction was stopped by addition of cold TCA to a final concentration of 5 percent. The measurements of incorporation of ^{14}C -glutamic acid into protein were made in a scintillation counter [(Aloka LSC-601) as described previously^{3,8}]. Four to five samples were observed at one point. The viability of blastocysts after incubation was estimated by nigrosin staining affinity¹⁴.

Results

Group I:—The value obtained in blastocysts recovered on day 5 to 6 of pregnancy is shown in Table 1. A tendency of increase in incorporation of ^{14}C -glutamic acid before implantation was observed in intact rats. The addition of glucose or lactate had an effect on incorporation of ^{14}C -glutamic acid ($P < 0.05$). These values between glucose and lactate were almost similar in each incubation time from 108 to 132 hour of pregnancy ($P > 0.05$).

Group II:—The value obtained in blastocyst recovered on day 5 to 6 of pregnancy is shown in Table 2. The alternation in the incorporation of glutamic acid from 108 to 132 hour of pregnancy was not observed in delayed blastocyst. The addition of glucose or lactate has not statistically increased the incorporation ($P > 0.05$).

Group III:—Administration of a single dose of 4 μ g estrone increased the incorporation of ^{14}C -glutamic acid compared with only progesterone treatment (Group II) during delayed implantation from day 5 to 6 of pregnancy ($P < 0.05$). These increases were about 1.5 to 3 times. The incorporation of ^{14}C -glutamic acid was maximum at 30 hour after estrone treatment. Addition of glucose or lactate in these estrone treatment increased the incorporation of ^{14}C -glutamic acid ($P < 0.05$), but addition of lactate has not statistically affected at the 108 hour of pregnancy ($P > 0.05$). The effect of addition of glucose and lactate was similarly observed in estrone treatment of each stage of pregnancy. These values in delayed implantation were lower compared with each stage of normal pregnancy ($P < 0.05$).

Table 1. Incorporation of ^{14}C -glutamic acid in blastocyst from day 5 to 6 of pregnancy of intact rats. (Mean $\pm$ S.E.) (cpm/egg)

	Hour of pregnancy					
	Addition of substrate		102	108	114	126
Group I	—		171.9 $\pm$ 10.3	206.2 $\pm$ 9.9	325.7 $\pm$ 13.5	430.8 $\pm$ 10.4
	glucose		355.6 $\pm$ 11.2	520.9 $\pm$ 12.4	651.3 $\pm$ 11.1	708.2 $\pm$ 15.4
	lactate		517.9 $\pm$ 13.4	581.4 $\pm$ 11.7	610.1 $\pm$ 10.3	656.5 $\pm$ 13.2

Table 2. Incorporation of ^{14}C -glutamic acid in delayed blastocyst from day 5 to 6 of pregnancy rats receiving daily 4 mg of progesterone, with or without 4 μg of estrone. (Mean $\pm$ S.E.) (cpm/egg)

	Hour of pregnancy					
	Addition of substrate		102	108	114	126
Group II Control (no estrone)	—		158.3 $\pm$ 9.7	202.1 $\pm$ 14.0	212.0 $\pm$ 12.4	210.7 $\pm$ 12.6
	glucose		161.6 $\pm$ 11.1	208.4 $\pm$ 14.8	250.7 $\pm$ 13.5	230.5 $\pm$ 14.2
	lactate		160.7 $\pm$ 10.3	204.5 $\pm$ 15.3	244.4 $\pm$ 9.9	242.1 $\pm$ 13.7
Group III Estrone	Hour after estrone treatment		0	6	12	24
	—		—	300.9 $\pm$ 12.2	312.3 $\pm$ 15.6	316.4 $\pm$ 15.2
	glucose		—	416.3 $\pm$ 7.8	480.7 $\pm$ 8.0	570.2 $\pm$ 14.8
	lactate		—	338.7 $\pm$ 11.6	470.5 $\pm$ 15.2	625.5 $\pm$ 18.9

Table 3. Incorporation of ^{14}C -glutamic acid in delayed blastocyst from day 8 to 9 of pregnancy rats receiving daily 4 mg of progesterone, with or without 4 μg of estrone. (Mean $\pm$ S.E.) (cpm/egg)

	Hour of pregnancy					
	Addition of substrate		172	178	184	196
Group IV Control (no estrone)	—		239.9 $\pm$ 8.9	252.2 $\pm$ 10.1	218.7 $\pm$ 7.5	211.7 $\pm$ 12.3
	glucose		258.8 $\pm$ 9.9	271.3 $\pm$ 13.2	239.7 $\pm$ 12.5	222.1 $\pm$ 9.6
	lactate		281.4 $\pm$ 12.1	258.7 $\pm$ 11.3	231.6 $\pm$ 8.9	229.4 $\pm$ 15.4
Group V Estrone	Hour after estrone treatment		0	6	12	24
	—		—	331.2 $\pm$ 13.7	380.1 $\pm$ 12.2	470.6 $\pm$ 15.7
	glucose		—	417.9 $\pm$ 10.3	561.1 $\pm$ 10.4	659.8 $\pm$ 10.7
	lactate		—	408.7 $\pm$ 9.9	541.8 $\pm$ 10.6	653.8 $\pm$ 12.8

Group IV:—The value obtained in blastocyst recovered on day 8 to 9 pregnancy is shown in Table 3. The incorporation of glutamic acid in blastocyst during delayed implantation was not different statistically from 172 to 196 hour of pregnancy ($P>0.05$). The values of 178 and 184 hour of pregnancy in the Group IV were higher than 102 and 108 hour of pregnancy in Group II ($P<0.05$). Addition of glucose or lactate had no effect on incorporation of ^{14}C -glutamic acid in the delayed implantation ($P>0.05$).

Group V:—Administration of a single dose of $4\text{ }\mu\text{g}$ estrone had an effect on incorporation of ^{14}C -glutamic acid in blastocyst during delayed implantation on day 8 to 9 of pregnancy ($P<0.05$). These incorporation were about 1.5 to 2 times the values of control (Group IV). Addition of glucose or lactate increased the incorporation of ^{14}C -glutamic acid in the case of estrone treatment during delayed implantation ($P<0.05$). The effects of addition of glucose and lactate on incorporation were almost similar at same hour of pregnancy as the case of estrone treatment ($P>0.05$). The value of 178 to 196 hour of pregnancy in delayed implantation were lower compared with blastocyst of day 5 to 6 pregnancy ($P<0.05$), but the value of 196 hour of pregnancy was not significantly different compared with 132 hour of pregnancy in the Group III ($P>0.05$).

The results from staining affinity of nigrosin have proved that the blastocysts survived during these incubation time.

Discussion

WEITLAUF *et al.*^{5,7)} have observed that the incorporation of ^{35}S -methionine in mice blastocyst decreases greatly during delayed implantation. The tendency of decrease in synthesis of DNA, RNA and protein (from ^3H -phenylalanine) of the rat blastocyst has also been observed during delayed implantation^{10,15-17)}. The present results demonstrate that protein synthesis of rat blastocysts, as inferred from the incorporation of ^{14}C -glutamic acid, is at a relatively high level on day 5 to 6 of normal pregnancy, but is at a low level in delayed implantation. This phenomenon indicates that blastocysts remain in a state of metabolic dormancy during delayed implantation. SANYAL *et al.*¹⁷⁾ have observed that the growth and differentiation of rat blastocyst is limited during delayed implantation. The length of major axis does exhibit little increase in delayed blastocyst¹⁸⁻²⁰⁾. Therefore, it seemed that these changes in growth and differentiation are related to the activity of DNA, RNA and protein synthesis in the delayed blastocyst. WEITLAUF *et al.*⁶⁾ have observed by the autoradiographical method that protein synthesis from ^{35}S -methionine in mice blastocyst of delayed implantation increased under the influence of estrogen. A tendency of great increase of the activities of DNA, RNA and protein synthesis (from ^3H -phenylalanine) in rat blastocysts has also been obtained by treatment of estrogen^{9,10,15,17,21)}. The present results have similarly showed that a single dose of $4\text{ }\mu\text{g}$ estrone given to ovariectomized pregnant rats increased the incorporation of ^{14}C -glutamic acid in delayed blastocyst. The administration of estrogen has produced the changes of trophoblast cells and the alterations in shape, axis lengths and area of delayed blastocysts^{19,20,22)}. The delayed blastocyst begins to induce the morphological changes within 12 to 24 hour and the implantation at 24 and 30 hour later, respectively, when estrone is administered on 5th day and 8th day of pregnancy¹⁹⁾. The number of nuclei is markedly increased 24 to 30 hour after administration of estrogen¹⁷⁾. WEITLAUF *et al.*⁶⁾ have observed that the blastocysts do not begin an appreciable protein synthesis until approximately 14 hour after the injection of estrogen in the mouse delayed blastocyst. In the present experiments, the incorporation of ^{14}C -glutamic acid increased at

6 hour after treatment of estrogen and increased markedly at 12 to 30 hour. The incorporation of ^{14}C -glutamic acid at 30 hour after administration of estrogen in the Group III was almost similar to the value at 126 hour of pregnancy in the Group I. Also, the value at 24 hour after administration of estrogen in the Group V was almost similar to the value at 126 hour of pregnancy in the Group I. Then, it is suggested that activities of the protein synthesis are related to the morphological changes in growth and differentiation of the blastocysts.

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ラット卵子の着床遅延時におけるグルタミン酸の 取込みに及ぼすエストロン投与の影響

辻井弘忠・菅原七郎・竹内三郎

東北大学農学部 家畜繁殖学教室, 仙台市

着床遅延時においてエストロンの単一投与がグルタミン酸の取込みに及ぼす影響について検討した。

スメア像に精子を見つけた日を妊娠第1日とし、妊娠3日目に去勢し、その後1日当りプロジェステロン4 mg/0.2 ml を連続注射した。受精後102時間目(妊娠

5日目), 172時間目(妊娠8日目)にそれぞれエストロン4 μg /0.2 ml を単一投与し、投与後6, 12, 18, 24, 30時間目のそれぞれの卵子をもちいて、グルタミン酸の取込みを調べた。なおコントロールとして正常妊娠5~6日目の卵子およびプロジェステロンのみを注射し

た場合の同時間に相当する卵子を用いた。測定の方法等は、前報と同様でグルタミン酸の濃度 $0.5 \mu\text{c/ml}$ (s. a. 0.11) として、グルコースまたは乳酸を添加し 37.5°C 下で、0, 60 分間培養し、液体シンチレーションで測定した。着床遅延時の卵子はエストロン投与によりグルタミン酸の取込みが、あきらかに増加することが認められた。またエストロン投与後 24, 30 時間目の卵子の取込

みは、正常妊娠 132 時間目の卵子より低い、それに近い取込みがみられた。前報で報告したように、グルコース、乳酸の添加により卵子のグルタミン酸の取込みを高め両者間に差が認められたが、着床遅延時の卵子においてはグルコース添加と乳酸添加との間に差はあまりみられなかった。